Ticks and Tick-borne Diseases 7 (2016) 216–223

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Original article

Siberian subtype tick-borne encephalitis virus in *Ixodes ricinus* in a newly emerged focus, Finland

Anu Jääskeläinen[a,*], Elina Tonteri[a], Ilkka Pieninkeroinen[b], Tarja Sironen[a], Liina Voutilainen[a,c], Markku Kuusi[d], Antti Vaheri[a,e], Olli Vapalahti[a,e,f]

[a] Department of Virology, University of Helsinki, P.O. Box 21, FI-00014 Helsinki, Finland
[b] Kymenlaakso Central Hospital, Kotkantie 41, FI-48210 Kotka, Finland
[c] Natural Resources Institute, Vantaa Unit, P.O. Box 18, FI-01301 Vantaa, Finland
[d] Department of Infectious Diseases, National Institute for Health and Welfare, P.O. Box 30, FI-00271 Helsinki, Finland
[e] Department of Virology and Immunology, HUSLAB, Helsinki University Hospital, P.O. Box 400, FI-00029 Helsinki, Finland
[f] Department of Veterinary Biosciences, University of Helsinki, P.O. Box 66, FI-00014 Helsinki, Finland

ARTICLE INFO

Article history:
Received 1 July 2015
Received in revised form
28 September 2015
Accepted 16 October 2015
Available online 19 October 2015

Keywords:
Tick-borne encephalitis virus
Tick-borne encephalitis
TBE
Siberian subtype
Tick
Ixodes ricinus

ABSTRACT

The first tick-borne encephalitis (TBE) cases in Kotka, Finland appeared in 2010. Altogether ten human cases have been diagnosed by 2014. Four had long-lasting sequelae.

We collected 195 *Ixodes ricinus* ticks, nine rodents, and eleven shrews from the archipelago of Kotka in 2011. Three Siberian subtype TBE virus (TBEV) strains were isolated from the ticks and three mammals were positive for TBEV antibodies.

The archipelago of Kotka is a newly emerged TBE focus of Siberian subtype TBEV circulating notably in *I. ricinus*. The patients had on average longer hospitalization than reported for the European subtype infection.

1. Introduction

Tick-borne encephalitis virus (TBEV) is the etiological agent of a potentially severe central nervous system infection, tick-borne encephalitis (TBE). TBE is endemic in Eurasia with thousands of human cases each year (Lindquist and Vapalahti, 2008). TBEV has three subtypes, the European (TBEV-Eur), the Siberian (TBEV-Sib), and the Far-Eastern (TBEV-FE) (Ecker et al., 1999), although revisions in taxonomy have been suggested (Grard et al., 2007).

In nature, TBEV circulates between ticks and small mammals. Once a tick becomes infected, it apparently stays infected and can transmit the virus further for the rest of its life (Gritsun et al., 2003a,b; Knap and Avšič-Županc, 2015). Rodents are reservoir hosts for TBEV and play an important role in TBEV transmission between ticks (Labuda and Randolph, 1999). The main tick vector species for TBEV-Eur is the castor bean tick, *Ixodes ricinus*, and for TBEV-Sib and TBEV-FE, the taiga tick, *I. persulcatus* (Lundkvist et al., 2001; Mavtchoutko et al., 2000).

The clinical symptoms of TBEV infection are unspecific and diagnosis has to be established in a laboratory. Usually patients seek for medical help only at the more severe second phase of the illness, when viral RNA has been cleared from the serum, and while IgM antibodies are usually present. Hence the diagnosis is based on detection of specific antibodies against TBEV (Holzmann, 2003). According to the case definition by the European Commission laboratory criteria for a confirmed TBE case include: (1) specific IgM and IgG in a single serum sample, (2) specific IgM in cerebrospinal fluid (CSF), (3) a significant increase in antibodies in paired serum sample, (4) detection of TBEV RNA, or (5) isolation of TBEV from a clinical sample (European Commission, 2012).

Finland is at the northern limit of the TBE endemic region in Europe and here TBE occurs focally. Few TBE endemic foci or areas have been known since 1960s, situated by the sea (the Åland Islands, and the archipelagos of Turku and Kokkola in western

* Corresponding author.
E-mail addresses: anu.jaaskelainen@helsinki.fi (A. Jääskeläinen), elina.rintala@helsinki.fi (E. Tonteri), ilkka.pieninkeroinen@fimnet.fi (I. Pieninkeroinen), tarja.sironen@helsinki.fi (T. Sironen), liina.voutilainen@helsinki.fi (L. Voutilainen), markku.kuusi@thl.fi (M. Kuusi), antti.vaheri@helsinki.fi (A. Vaheri), olli.vapalahti@helsinki.fi (O. Vapalahti).

http://dx.doi.org/10.1016/j.ttbdis.2015.10.013
1877-959X/

Finland) or by big lakes (around Lappeenranta in south-eastern Finland) (Tuomi and Brummer-Korvenkontio, 1965). Other foci have been detected since: Isosaari island in the archipelago of Helsinki in the 1990s (Han et al., 2001) and Simo at the northern coast of Bothnian Gulf since 2008 (Jääskeläinen et al., 2011). Sporadic human cases have been reported from several other locations, all by the sea or big lakes, in the 2000s and 2010s (Tonteri et al., 2015).

The two first human TBE cases ever diagnosed in the archipelago of Kotka at the Gulf of Finland in 2010 led us to conduct this study. We collected ticks and small mammals from the most likely sites of infections and studied them for the presence of TBEV infection markers. Since then, additional human cases have occurred in Kotka archipelago. We describe TBE cases infected in the Kotka archipelago from 2010 to 2014, and report determination of the tick species, prevalence of TBEV in the ticks, virus isolation in cell culture, and genetic characterization of the isolates including finding an unexpected TBEV subtype considering the tick species involved.

2. Materials and methods

2.1. TBE patients and serological tests

Finnish TBE cases are notified by clinical microbiology laboratories to the Infectious Diseases Register (2015) maintained by the National Institute for Health and Welfare. The information on the localities from where TBE patients have acquired their infection is based on medical reports of the patients and interviews done for the patients by the National Institute for Health and Welfare. Ten cases in 2010–2014 were from Kotka archipelago.

The patient serum and CSF samples were studied for TBEV antibodies at HUSLAB, Helsinki University Hospital, by an in-house μ-capture IgM EIA test (Jääskeläinen et al., 2003) and total antibodies were determined by an in-house hemagglutination inhibition test (details described in Jääskeläinen et al., 2003). The ethical permit to study the patients' clinical records was given by HUSLAB (permission number TYH2014251).

2.2. Ticks and small mammals

We collected questing ticks ($N = 195$) by flagging and small mammals ($N = 20$) using snap-traps on three islands (Haapasaari, Kirkonmaa, and Sisä-Nuokko, Fig. 1), that were the most likely sites of infections of the patients in 2010, in the archipelago of Kotka in June 2011 (Table 1). The tick species was defined by morphology according to Filippova (1977), and by PCR for the TBEV positive pools and one negative pool (Rumer et al., 2011). The ticks were stored alive at +4 °C until homogenization in 500 μl Dulbecco's phosphate-buffered saline (PBS) with 0.2% bovine serum albumin. The adults were homogenized in pools of two, and the nymphs in pools of 4–10, in altogether 67 pools. Homogenized aliquots were stored in −70 °C until nucleic acid extraction and virus isolation experiments.

The small mammals (Table 1) were set in dry ice when collected and stored at −80 °C until dissection.

2.3. RNA and DNA extraction, TBEV-RT-PCR, tick species PCR, and IFA

RNA was extracted from aliquots of 100 μl of homogenized tick pools or lung and brain samples of small mammals by TRIsure™ (Bioline) according to the manufacturer's instructions. Presence of TBEV genome in tick pools and in mammal organ samples was screened using a real-time RT-PCR (Schwaiger and Cassinotti, 2003). Samples positive in real-time RT-PCR were confirmed by 5′NCR-RT-PCR (Schrader and Süss, 1999). From TBEV-positive tick samples as well as from one negative tick sample, also DNA was extracted, according to the instructions provided for TriPure Isolation Reagent (Roche Applied Sciences), and the tick species was further confirmed by molecular analyses according to Rumer et al. (2011). The PCR products were purified by a PCR Purification Kit (Qiagen) and T/A-cloned to pGEM-T vector (Promega). One to four clones of each tick pool were sequenced with SP6 and T7 primers. Sequencing was performed by Sanger sequencing.

For serological analysis the hearts of rodents were rinsed with 300 μl and hearts of shrews by 200 μl of PBS to elute the blood from them (corresponding to approximately 1:10 serum dilution). Samples were analyzed using an immunofluorescence assay (IFA) with acetone-fixed TBEV-infected (strain Kumlinge A52) cells mixed with uninfected (1:1) Vero E6 cells (ATCC CRL-1586) as antigen.

2.4. Virus isolation

TBEV-RNA positive tick pools were applied to virus isolation experiments on Vero E6 cell cultures (Achazi et al., 2011) and subjected to nine sequential blind passages. A third of the cells were always passaged further, and the remaining cells were used for detection of TBEV antigen, which was screened by IFA with the monoclonal antibody 1493 targeting the TBEV E protein (Niedrig et al., 1994) at 2.5 μg/ml.

One hundred forty microliters of supernatants of the passages was applied to RNA extraction by QIAamp Viral RNA Mini Kit (Qiagen) according to manufacturer's instructions for screening for TBEV RNA as above.

2.5. Determination of partial E and NS5 sequences

Based on RT-PCR and antigen results, one supernatant from each isolated strain was chosen for further experiments. The extracted RNA was transcribed to cDNA by random hexamers and Expand RT enzyme (Roche Diagnostics GmbH). Partial E gene was amplified by TBEV-E primers (Jääskeläinen et al., 2006) using Phusion Flash Master Mix (Thermo Scientific) with the following PCR program: initial denaturation at 98 °C for 10 s, then 36 cycles of 98 °C for 1 s, 65 °C for 5 s and 72 °C for 25 s, and final extension at 72 °C for 1 min. The PCR products were purified by a PCR Purification Kit (Qiagen) and

Table 1
Ticks and small mammals from Kotka archipelago 2011.

Island	Ticks/pools (F/M/N)	TBEV RNA pos	*M. gla*/TBEV ab pos/total	*M. agr*/TBEV ab pos/total	*A. amp*/TBEV ab pos/total	*S. ara*/TBEV ab pos/total
Haapasaari	156/49 (24/58/74)	3	0/0	0/2	1/1	0/8
Kirkonmaa	36/16 (14/15/7)	0	0/0	0/2	0/0	0/3
Sisä-Nuokko	3/2 (0/2/1)	0	2/3	0/1	0/0	0/0

F, females; M, males; N, nymphs. *M. gla*, *Myodes glareolus*, bank vole; *M. agr*, *Microtus agrestis*, field vole; *A. amp*, *Arvicola amphibius*, water vole; *S. ara*, *Sorex araneus*, common shrew; TBEV ab pos, TBEV antibody positive.